

Biochemistry

CHARACTERIZATION OF PROTEIN S-NITROSOTHIOLS

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S-nitrosothiols (SNOs) are metabolites of nitric oxide (NO) and are involved in signal transduction. When RAW 264.7 macrophage cells are treated with spermine NONOate in an oxygen environment, protein SNOs are formed. The amount of SNOs was determined through an I_3^- -dependent chemiluminescence assay and normalized against total protein content. Protein SNOs were characterized by molecular weight and protein charge. Regarding molecular weight, protein SNOs were filtered by through 3 kDa, 10 kDa, and 50 kDa Microcon cut-off filters. SNOs were detected in the raw lysate but not in the resulting filtrates, demonstrating that protein SNOs have a molecular weight greater than 50 kDa. Regarding protein charge, cell lysate was separated into positively and negatively charged protein fractions by DE 52 Microgranular Anion Exchange resin. It was found that more protein SNOs were present in the positively charged protein fraction than in negatively charged protein fraction. These results indicate an uneven distribution of SNOs among the proteins of the cell, which could be used to characterize the specific protein or proteins that contain SNOs.